Journal of Medical Virology 57:9–16 (1999)

# Induction of Intercellular Adhesion Molecule 1 Gene Expression by Measles Virus in Human Umbilical Vein Endothelial Cells

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The expression of intercellular adhesion molecule 1 (ICAM-1) by endothelial cells is important for the regulation of adhesion and transendothelial migration of a variety of leukocytes that express the integrins lymphocyte function-associated antigen 1 (LFA-1) and/or Mac-1. Here, we demonstrate strain-specific differences in the ability of measles virus (MV) to induce ICAM-1 expression. The vaccine strain Moraten (Mor) rapidly induced high levels of ICAM-1 mRNA and protein expression, whereas the vaccine strain CAM-70 and the Edmonston wild type (Ed-wt) strain were far less effective, even when they were used at very high multiplicities of infection (MOIs). Strain-specific differences in the induction were not a consequence of differences in the ability to infect ECs. Furthermore, induction of ICAM-1 by Mor was not dependent on de novo expression of MV or cellular proteins. Dual-immunofluorescence analysis indicated that there was no association between the expression of either MV nucleocapsid or hemagglutinin protein and the induction of ICAM-1 expression. Some human umbilical vein endothelial cells (HUVECs) that expressed high nucleocapsid protein in response to either Mor or CAM-70 failed to express elevated ICAM-1, whereas some HUVECs that were incubated with Mor expressed high ICAM-1 prior to expression of MV nucleocapsid protein. Strain-specific differences in the ability of Mor and CAM-70 to induce ICAM-1 correlated with their ability to activate the latent transcription factor NF-κB. These studies suggest a preexisting component of MV particles that leads to strain-specific differences in the activation of NF-κB and the induction of ICAM-1 gene expression. ***J. Med. Virol. 57:9–16, 1999.*** 

**KEY WORDS:** cellular adhesion molecules; vaccines; double-stranded RNA; NF-κB

## INTRODUCTION

Measles virus (MV) is an enveloped, negative-stranded RNA virus with a nonsegmented genome that belongs to the family *Paramyxoviridiae* [Bellini et al., 1994]. MV infection is responsible for the deaths of an estimated 1 million children every year, more deaths world wide than from all other childhood vaccine-preventable diseases combined [Gellen and Katz, 1994a,b]. The vast majority of these deaths occur in unvaccinated children living in Third World countries. In the United States and in other developed countries, there is a high vaccine coverage rate; however, outbreaks still occur.

Endothelial cells are among the cells that are infected with MV. Electron microscopy has shown MV nucleocapsids in the endothelial cells of dermal capillaries taken from skin biopsies of infected individuals at the preeruptive and early eruptive stages of the maculopapular rash [Kimura et al., 1975a,b]. Biopsies also display extensive leukocyte infiltration into the areas adjacent to the infected endothelial cells, suggesting that MV may induce cellular adhesion molecules to mediate this effect directly or indirectly. Indeed, MV has been reported recently to induce intercellular adhesion molecule 1 (ICAM-1) expression on

Grant sponsor: National Institutes of Health; Grant numbers: RO1 CA60345 and P30AR42687.

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Accepted 8 July 1998

endothelial cells [Soilu-Hanninen et al., 1996], but it was not determined whether induction was dependent on direct infection of the cells or on a secondary event. ICAM-1 is an important cellular adhesion molecule that is involved in this process. Although it is expressed constitutively at low levels on endothelial cells, ICAM-1 can be induced to high levels of expression by proinflammatory agents, such as tumor necrosis factor alpha (TNF-$\alpha$), interleukin 1 beta (IL-1$\beta$), interferon gamma (IFN-$\gamma$), lipopolysaccharide (LPS), and the synthetic double-stranded RNA, polyriboinosinic:polyribocytidylic acid [poly(I:C)] [Caughman et al., 1990, 1992; Deigtz et al., 1991; Dustin et al., 1986; Yang et al., 1994]. The integrin lymphocyte function-associated antigen 1 (LFA-1), which is expressed on lymphocytes and monocytes, and the integrin Mac-1, which is expressed on polymorphonuclear cells, serve as counterreceptors for ICAM-1. The interaction between ICAM-1 and LFA-1 and/or Mac-1 regulates in part the tight adhesion and transendothelial migration that leads to leukocyte infiltration into an area of inflammation [Dustin and Springer, 1988; Simmons et al., 1988].

In this study, we demonstrate that Moraten (Mor), a vaccine strain of MV, induces ICAM-1 mRNA and protein expression in human umbilical vein endothelial cells (HUVECs), whereas another effective vaccine, CAM-70, induces low levels of ICAM-1 only when it is used at a high multiplicity of infection (MOI). The relationship between viral gene expression and ICAM-1 induction is explored.

## MATERIALS AND METHODS

### Cell Culture and MV Strains

HUVECs were isolated from human umbilical veins and were grown under conditions that have been described previously [Zimring et al., 1998]. Cells were passaged at confluency by splitting 1:4, and cells were used within the first eight passages.

The Mor vaccine strain of MV (Attenuvax; Merck, Sharp, and Dohme, West Point, PA) was derived originally from a wild type MV [Enders, 1954; Rota et al., 1994]. Virus was propagated at a MOI in Vero E6 African green monkey kidney cells in Eagle's minimal essential medium (EMEM; Gibco BRL, Gaithersburg, MD) with 2% fetal bovine serum (FBS; Hyclone Laboratories, Inc., Logon, UT). The CAM-70 vaccine strain of MV was derived from the Tanabe wild type virus [Rota et al., 1994] and was propagated in a manner similar to that used for Mor. A low-passage seed stock of the Edmonston wild type (Ed-wt) strain of MV was obtained and grown as described previously [Rota et al., 1994]. Either an uninfected Vero cell lysate diluted in the same manner as the virus or serum-free media was used as a control inoculum. Comparable levels of ICAM-1 expression resulted from either control. Virus titers were determined with a plaque assay by using serially diluted virus on Vero cells. After adsorption, 1 ml agar overlay (1:1 mixture of 2% SeaPlaque agarose; FMC BioProducts, Rockland, ME) and 2 × modified Eagle's medium (Life Technologies, Grand Island, NY) supplemented with 4% FBS, 100 U/ml penicillin, and 100 U/ml streptomycin) were added. Five days postinfection (p.i.), neutral red was added for 24 hours, and the plaques were counted.

### Incubation Conditions With Virus

The concentrations of virus indicated in each experiment were added to M199 medium with no supplements in a final volume of 2 ml per P150 plate and incubated at 37°C for 1 hour with shaking every 15 minutes. After 1 hour, 13 ml of fully supplemented M199 medium were added to each plate for the amount of time indicated in each experiment. For experiments using 96-well plates, the amount of virus in M199 medium used for infection was 15 $\mu$l, followed by 185 $\mu$l of fully supplemented M199 medium. Mock-infected conditions were done by using an equal volume of uninfected Vero cell lysate.

### RNA Isolation and Northern Blot Analysis

Total cellular RNA was isolated by using RNAsol (Tel-Test Inc., Friendswood, TX) according to the manufacturer's instructions. Total cellular RNA (15–20 $\mu$g) was size fractionated by using 1% agarose formaldehyde gels in the presence of 1 $\mu$g/ml ethidium bromide [Selden, 1987]. The RNA was transferred to nitrocellulose (Gibco BRL), was linked covalently by ultraviolet (UV) irradiation using a Stratalinker UV cross linker (Stratagene, La Jolla, CA), and was then baked for 2 hours at 80°C. After a 4-hour prehybridization in the absence of $^{32}$P-labeled DNA or dextran, nitrocellulose was hybridized at 42°C overnight in a solution containing 5 × standard saline citrate (SSC), 1% sodium dodecyl sulfate (SDS), 5 × Denhardt's solution, 100 $\mu$g/ml salmon sperm DNA, 50% formamide, 10% dextran sulfate, and approximately 100 ng $^{32}$P-dCTP DNA (ICN Biomedicals, Costa Mesa, CA) labeled to a specific activity of $1 \times 10^8$ cpm/$\mu$g. DNA probes were made by using the random primer oligonucleotide method with the Oligolabeling Kit from Pharmacia Biotech (Piscataway, NJ). Blots were washed twice in 2 × SSC/1% SDS for 30 minutes at 55°C then once in 0.2 × SSC/0.1% SDS for 30 minutes at 55°C. Autoradiography was performed with an intensifying screen at −70°C. Blots were stripped by using boiling water prior to rehybridization with additional probes. The ICAM-1 probe was a 1.8-kb *EcoR*I fragment of human cDNA [Staunton et al., 1988]. The MV nucleocapsid gene probe was a 1.7-kb *Xho*I/*EcoR*I fragment containing the full-length gene. The glyceraldehyde 3-phosphate dehydrogenase probe was a 1.2-kb *EcoR*I fragment of the human cDNA excised from clone HHCMC32 [Adams et al., 1992].

### Flow Cytometric Analysis

HUVECs were rinsed twice with phosphate-buffered saline (PBS); detached by using 5 ml versene with gentle scraping; rinsed once with PBS and once with 0.01 M Ca- and Mg-free PBS, pH 7.4 plus 1% bovine serum albumin plus 0.1% sodium azide (FACS buffer);

and dispensed into aliquots containing approximately $2 \times 10^5$ cells each. Samples were fixed in fixation buffer [4% EM grade paraformaldehyde (Ted Pella, Inc., Redding, CA) in Ca- and Mg-free PBS] at 4°C for 20 minutes. Samples were washed once in permeabilization buffer (FACS buffer plus 0.1% saponin) then resuspended in 20 μl permeabilization buffer for 10 minutes at room temperature. Twenty microliters of a monoclonal antibody to the MV nucleocapsid protein, KK2 [Bellini et al., 1986], diluted 1:250 in permeabilization buffer were added for 30 minutes at 4°C. After incubation, the samples were rinsed with cold permeabilization buffer and incubated for 30 minutes at 4°C with phycoerythrin (PE)-conjugated goat antimouse immunoglobulin G (IgG) antibody (Sigma Chemical Company, St. Louis, MO) diluted 1:20 in permeabilization buffer. After incubation, the samples were rinsed with cold permeabilization buffer and incubated for 30 minutes at 4°C with fluorescein isothiocyanate (FITC)-conjugated mouse-antihuman ICAM-1 antibody (Ancell Corporation, Bayport, MN) diluted 1:50 in permeabilization buffer. After incubation, the samples were washed in permeabilization buffer and resuspended in FACS buffer. The samples were then analyzed by flow cytometry on a FACSort (Becton-Dickinson, San Jose, CA).

### Enzyme-Linked Immunosorbent Assays for Cellular Adhesion Molecule Expression

Cell surface ICAM-1 expression was assayed on confluent live cells by incubation with mouse antihuman ICAM-1 antibody 84H10 diluted to 1 μg/ml in complete media for 30 minutes at 37°C. Wells for background determination were included in which no primary antibody was used. Cells were washed and then incubated for 30 minutes with peroxidase-conjugated goat-antimouse IgG antibody (BioRad, Richmond, CA) diluted 1:1,000 in medium. Cells were then washed three times with PBS to reduce nonspecific binding of the secondary antibody. Binding of the secondary antibody was assessed by addition of 100 μl of 0.1 mg/ml tetramethylbenzidine (Sigma Chemical Co.) and 0.01% $H_2O_2$. The reaction was stopped by the addition of 25 μl of 8 N sulfuric acid, and the plates were read on a BioRad reader (model 450 ELISA Reader) at 450 nm. Quadruplicate conditions were done for the calculation of standard deviation.

### Electrophoretic Mobility Shift Assays

Electrophoretic mobility shift assay (EMSA) analysis was performed by using 5 μg per lane of nuclear extract, as described previously [Offermann et al., 1995; Zimring et al., 1998].

## RESULTS

To determine whether MV induced ICAM-1 expression on the surface of HUVECs, cells were infected with Mor, and ICAM-1 levels were measured by indirect enzyme-linked immunosorbent assay 24 hours p.i. ICAM-1 was induced fourfold and twofold in Mor-infected cells by using MOIs of 12 and 1, respectively, but was not elevated in cells infected at a lower MOI (Fig. 1A). ICAM-1 induction by Mor at an MOI of 12 was comparable to the level of induction by poly(I:C), a synthetic, double-stranded RNA that has been shown to be a powerful inducer of ICAM-1 [Yang et al., 1994], and Mor at an MOI of 1.0 induced ICAM-1 levels to approximately 50% of that induced by poly(I:C). Although virus was used routinely as an unfractionated infected Vero cell lysate, the supernatant fraction obtained after removal of the virus from the Vero cell lysate by ultracentrifugation induced ICAM-1 only minimally (data not shown). In contrast, CAM-70, which is an effective vaccine derived from a Japanese wild type strain, did not induce ICAM-1 expression on HUVECs when it was used at MOIs of 1.0 or 12, but it did induce ICAM-1 2.5-fold when it was used at an MOI of 40 (Fig. 1B). Thus, there were strain-specific differences in the ability of live-attenuated vaccine strains of MV to induce ICAM-1.

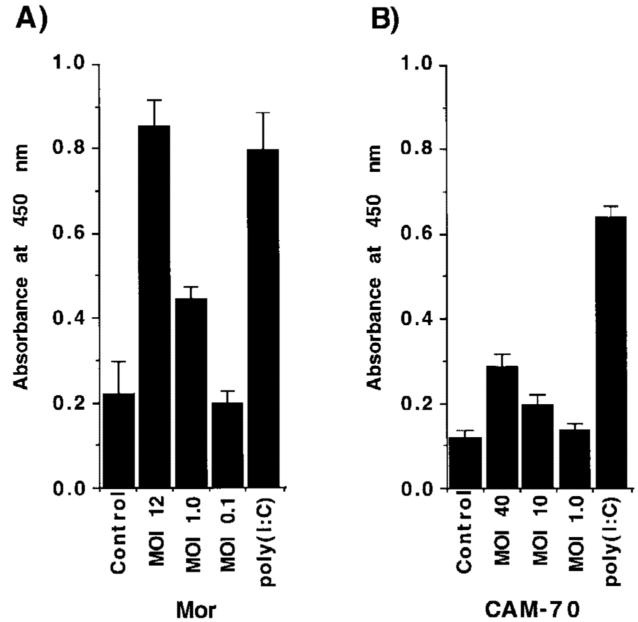


Fig. 1. Induction of intercellular adhesion molecule 1 (ICAM-1) on human umbilical vein epithelial cells (HUVECs) by Moraten (Mor) and CAM70. HUVECs were infected with Mor (**A**) or CAM-70 (**B**) at the indicated multiplicities of infection (MOIs). ICAM-1 protein levels were measured at 24 hours postinfection (p.i.) by using enzyme-linked immunosorbent assay (ELISA). Poly(I:C) was used at a concentration of 100 μg/ml. An uninfected Vero cell lysate was used as control inoculum in A, whereas serum-free medium was used as control inoculum in B. Values shown are the average absorbance levels measured at 450 nm of quadruplicate wells ± standard deviation.

To determine whether the observed changes in ICAM-1 surface expression were detectable at the mRNA level, Northern blot analysis was performed. ICAM-1 mRNA was detected at 6 hours p.i. (data not shown), and ICAM-1 levels remained elevated at 24 hours and 72 hours p.i. with Mor (Fig. 2). In contrast, CAM-70 infection did not induce ICAM-1 mRNA 24 hours p.i., but it did induce low amounts of ICAM-1

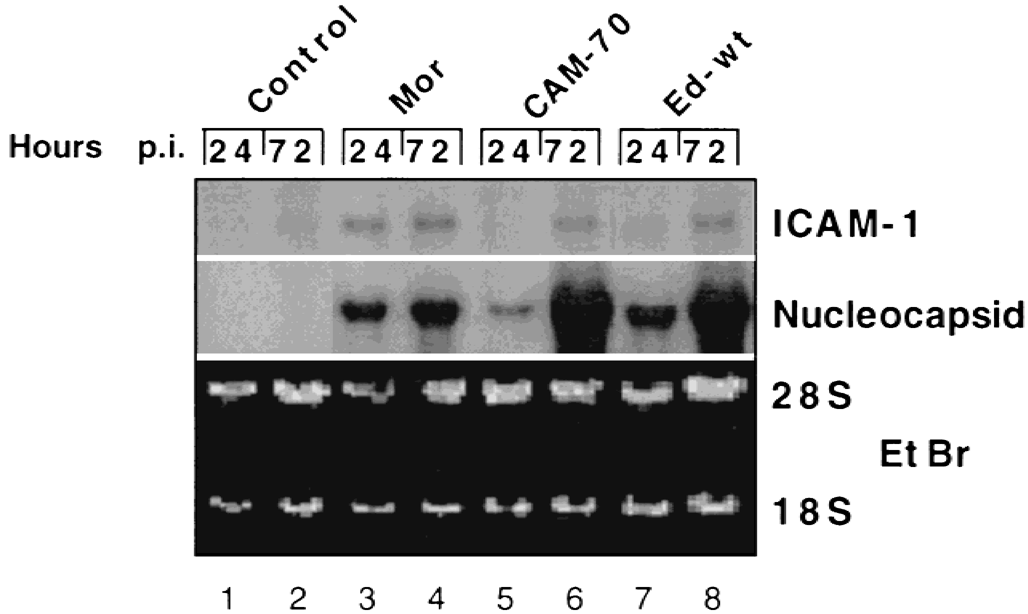


Fig. 2. **Lanes 1–6:** Changes in ICAM-1 and nucleocapsid gene mRNA in response to Mor, CAM-70, and Edmonston wild type strain (Ed-wt). HUVECs were infected with Mor, CAM-70, or Ed-wt at an MOI of 1.0, and RNA was isolated from all cells. Serum-free medium was used as control inoculum. Total cellular RNA (20 μg) was size fractionated and analyzed by Northern blot analysis. All bands visualized by autoradiography coincided with the known size of the mRNA of each gene. Ethidium bromide levels are shown for comparison of RNA loads between lanes.

mRNA by 72 hours p.i. In addition, the Ed-wt strain was used to investigate ICAM-1 induction by a wild type strain. The pattern of ICAM-1 induction and nucleocapsid gene expression by Ed-wt was similar to that observed for CAM-70.

To determine whether differences in the increase in ICAM-1 were a consequence of differences in viral transcription, expression of the MV nucleocapsid gene was examined. A double-stranded DNA probe that was used to detect nucleocapsid mRNA also detected high-molecular-weight species (50S) representing the genomic and antigenomic RNA (data not shown). Infection with Mor or Ed-wt produced significantly more nucleocapsid mRNA than CAM-70 by 24 hours p.i. (Fig. 2, lanes 3, 5, and 7). By 72 hours p.i., the amount of nucleocapsid mRNA produced in CAM-70- and Ed-wt-infected cells was dramatically higher than in Mor infected cells (Fig. 2, lanes 4, 6, and 8), but there was more ICAM-1 mRNA in response to Mor. Thus, the ability of Mor to induce earlier and higher ICAM-1 mRNA levels than CAM-70 or Ed-wt was not a consequence of increased viral transcription. The greater level of nucleocapsid mRNA transcription that occurred from CAM-70 and Ed-wt was associated with less ICAM-1 mRNA.

To determine the relationship between viral nucleocapsid (N) protein expression and ICAM-1 expression, dual-immunofluorescence flow cytometry was carried out. At 24 hours p.i., with Mor at an MOI of 1.0, we detected three major subsets of the population of cells (Fig. 3B). One subset comprising 6.9% of all cells expressed high levels of N-protein and basal levels of ICAM-1, whereas a second subset consisting of 7.3% of all cells expressed increased levels of ICAM-1 without increased levels of N protein. Fewer cells (3.8%) had increased ICAM-1 and N protein, and the remaining subset did not have increased levels of ICAM-1 or N protein. These data indicated that the induced ICAM-1 was not directly associated with viral protein expression. When Mor was added at an MOI of 4.0, ICAM-1 levels increased globally, but the majority of the cells that expressed high levels of ICAM-1 (21.6%) did not express high levels of N protein (Fig. 3C). Only 9.4% had both elevated ICAM-1 and N protein, and 7.5% expressed high levels of N protein without increased levels of ICAM-1. This pattern of induction further illustrates that detectable N-protein expression was not associated directly with ICAM-1 induction. A similar analysis was undertaken with CAM-70, a strain that induced ICAM-1 only weakly at high MOIs. Despite very high levels of N-protein expression in over 50% of the cells infected with CAM-70, 90% of them did not display elevated levels of ICAM-1 (Fig. 3D). Therefore, for both Mor and CAM-70, increased N protein expression did not correlate with increased ICAM-1 expression. Similar results were obtained when viral hemagglutinin protein expression was compared with ICAM-1 expression (data not shown).

The dual-immunofluorescence data suggested that ICAM-1 induction was not dependent on expression of viral proteins. To determine whether the induction of ICAM-1 by Mor occurred independent of de novo protein synthesis, cells were treated with cycloheximide (Cx) to block protein synthesis and were infected with either Mor or CAM-70 at an MOI of 1.0. RNA was harvested, and MV nucleocapsid or ICAM-1 mRNA was

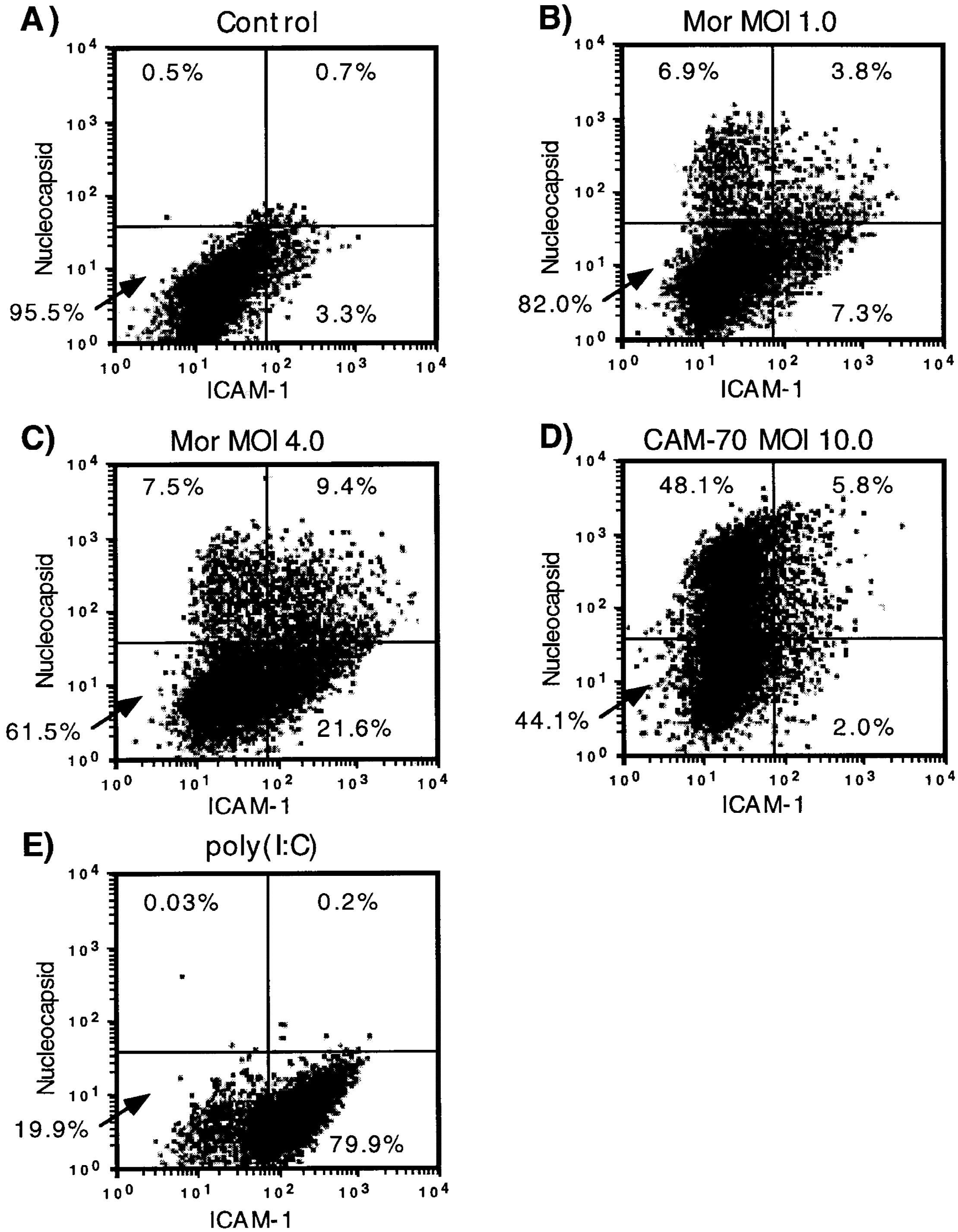


Fig. 3. ICAM-1 and nucleocapsid protein expression in Mor-and CAM-70-infected HUVECs. Dot plots are shown of dual-immunofluorescence flow cytometry performed on uninfected HUVEC controls (**A**), on Mor-infected HUVECs at an MOI of 1.0 (**B**) or 4.0 (**C**), on CAM-70-infected HUVECs at an MOI of 10.0 (**D**), or on HUVECs treated with polyriboinosinic:polyribocytidylic acid [poly(I:C)] at 100 μg/ml (**E**) for 24 hours. Serum-free medium was used as control inoculum. ICAM-1 immunofluorescence is presented along the x-axis, whereas N-protein immunofluorescence is presented along the y-axis. Boundaries for ICAM-1 basal expression were based on uninfected cells in which 95.5% of constitutive ICAM-1-expressing cells were included, and 99% of the control cells lacked N-protein signal. Percentages in the quadrants represent the percentage of cells falling into that quadrant out of 10,000 events detected.

detected by Northern blot analysis. Although both Mor and CAM-70 displayed comparable levels of nucleocapsid mRNA expression, ICAM-1 mRNA was induced only by Mor and not by CAM-70 by 8 hour p.i. (Fig. 4, lanes 2 and 3, respectively). Incubation with Cx alone induced ICAM-1 mRNA expression (Fig. 4, lane 5). However, Mor infection in the presence of Cx caused a superinduction of ICAM-1 mRNA (Fig. 4, lane 6). This

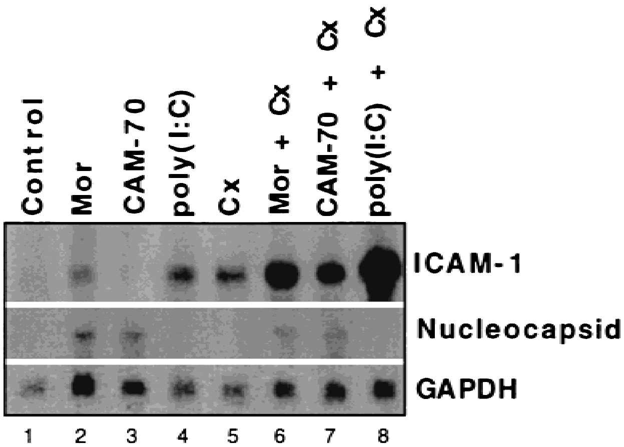


Fig. 4. Effect of cycloheximide (Cx) on ICAM-1 and nucleocapsid gene mRNA induction. HUVECs were infected with Mor or CAM-70 at an MOI of 1.0 or were treated with poly(I:C) at 100 μg/ml in cells that were either pretreated for 30 minutes with control medium or with medium containing 10 μg/ml Cx. Cx was present for the duration of the experiment. Serum-free medium was used as control inoculum. Total cellular RNA was isolated from cells that were harvested 8 hours p.i. Total cellular RNA (15 μg) was size fractionated and analyzed by Northern blot analysis. All bands visualized by autoradiography coincided with the known size of the mRNA of each gene. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) levels were used to control for RNA loading between lanes.

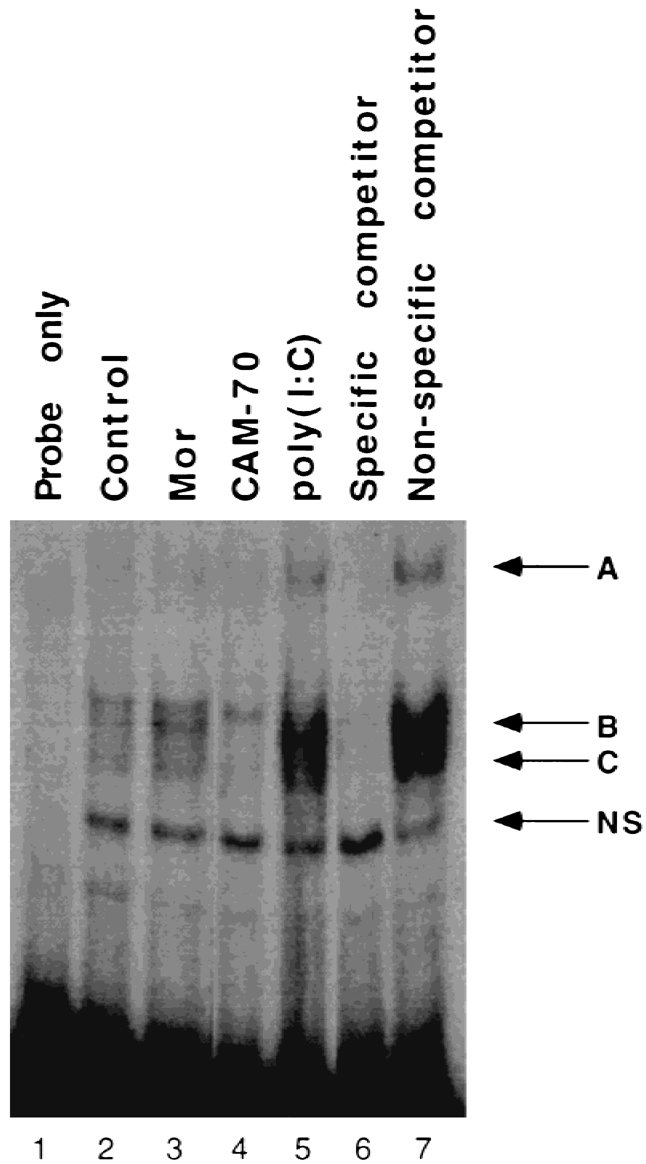


Fig. 5. Activation of NF-κB by Mor and CAM-70. HUVECs were infected with Mor or CAM-70 at an MOI of 1.0 or were treated with poly(I:C) at 100 μg/ml for 7 hours and then harvested for nuclear protein. Serum-free medium was used as control inoculum. Gel shift analysis was performed using 5 μg of nuclear protein per sample using a $^{32}$P-labeled probe consisting of two NF-κB binding sites from the VCAM promoter [Offermann et al., 1995]. Complexes A–C are specific binding complexes, whereas complex NS is a nonspecific binding complex. Cold specific competitor was added in 30-fold excess, and the nonspecific competitor, the positive regulatory domain I (PRDI) site from the interferon beta promoter was also added in 30-fold excess. Binding complexes were resolved by using a 4% polyacrylamide gel and were visualized with autoradiography.

superinduction was less than the superinduction observed in cells treated with poly(I:C) and Cx (Fig. 4, lane 8). It is interesting to note that, although CAM-70 infection did not induce ICAM-1 expression, CAM-70 infection in the presence of Cx resulted in a superinduction of ICAM-1 (Fig. 4, lane 7), but the superinduction was less than that what occurred in response to Mor.

The ICAM-1 promoter is regulated in part by the transcription factor NF-κB [Collins et al., 1995]. We hypothesized that differences in the ability of Mor and CAM-70 to induce ICAM-1 might result from differences in the ability to activate NF-κB. NF-κB activation was assayed by using gel-shift analysis. We observed activation of NF-κB 7 hours p.i. with Mor but not with CAM-70 (Fig. 5, lanes 3B,C and 4, respectively). The levels of NF-κB activation in response to Mor infection were less than in poly(I:C) treated cells. Thus, the differences in NF-κB activation after infection by Mor or CAM-70 correlated with differences in the ability of these viruses to induce ICAM-1.

## DISCUSSION

We have demonstrated that there are strain-specific differences in the induction of ICAM-1 by two vaccine strains of MV, with Mor effectively inducing ICAM-1, whereas CAM-70 led to only marginal increases in ICAM-1 despite producing higher levels of expression of the nucleocapsid gene mRNA than Mor. In addition, the pattern of ICAM-1 induction and nucleocapsid gene expression by Ed-wt was similar to that observed for CAM-70. In addition to Ed-wt, we have found that several other wild-type strains of MV differ in their ability to induce ICAM-1 [Harcourt et al., 1995; unpublished observations]. The strain-specific differences in the induction of ICAM-1 correlated with strain-specific differences in the activation of the transcription factor NF-κB. Moreover, the observed differences were not merely a consequence of differences in the ability of these strains to infect endothelial cells, and the induction of ICAM-1 mRNA by Mor neither correlated with

N protein or hemagglutinin protein expression nor was dependent on de novo protein synthesis.

The mechanism for the differential induction of ICAM-1 by Mor, CAM-70, and Ed-wt is unknown. Because N protein is the first protein that is expressed following infection, other MV proteins are unlikely to be expressed in the absence of N protein. Thus, either a transcriptional event, a preexisting protein in the MV particle, or a nonprotein component most likely is responsible for the induction of ICAM-1 by Mor. In fact, dsRNA is a potent activator of NF-κB and inducer of ICAM-1 in HUVECs [Marui et al., 1993; Offermann et al., 1995], and the viruses may differ in their ability to generate biologically active dsRNA intermediates. Also, strain-specific differences could result from variations in the secondary structure of MV RNA that could occur in genomic RNA, in mRNAs, or in noncoding regions, such as intergenic sequences or leader and trailer sequences. Genetic analysis of the Mor and CAM-70 nucleocapsid, hemagglutinin, and fusion genes revealed that Mor and CAM-70 are in the same genetic group. Compared with the prototype Ed-wt isolate, there are 26 nucleotide changes resulting in 19 amino acid changes in CAM-70, whereas Mor contained 13 nucleotide changes resulting in eight amino acid changes in the 6,000 bases that comprise the H, F, and N genes [Rota et al., 1994]. At present, it is impossible to predict what effect these nucleotide or amino acid substitutions may have on the induction of ICAM-1. Additional sequencing of the Mor and CAM-70 viruses, particularly the regions that contain the intergenic sequence and the leader and trailer sequences, may be more informative. It is possible that the differences in ICAM-1 induction are due to variations in the ratio of infectious to noninfectious MV particles in the viral stocks. Although viral stocks were prepared by using protocols that were designed to minimize the generation of defective interfering particles, the possibility that the viral preparations may have contained varying amounts of these particles cannot be ignored.

The increase in ICAM-1 levels on cells that do not express N protein is unlikely to be due solely to a soluble factor secreted by MV-infected cells, because such a mechanism would not explain the lack of ICAM-1 induction on many of the cells expressing MV-proteins or the superinduction that occurs in the presence of Cx. Furthermore, soluble factors would be expected to cause more uniform changes in ICAM-1 expression than those that were observed here.

The vascular bed of origin of the endothelial cells affects responsiveness [Swerlick et al., 1992; Cornelius et al., 1993], and transformed or immortalized cells generally express lower levels of ICAM-1 than nontransformed cells [Ades et al., 1992]. Thus, MV strain differences as well as the use of transformed endothelial cells may account for the low level of ICAM protein induction that was reported previously in a transformed endothelial cell line using the Halonen wild type strain [Soilu-Hanninen et al., 1996], and species differences may account for the lack of ICAM-1 induction by MV in BALB/c mice brain microvascular endothelial cells [Brankin et al., 1995].

Although MV enters the host through the respiratory tract, one hypothesis for its dissemination is through infected monocytes [Gellin and Katz, 1994a]. Some, but not all, strains of MV induce higher levels of LFA-1 expression on infected monocytes [Attibele et al., 1993; Wyde et al., 1994; Hummel et al., 1998]. It is interesting to note that Mor induces LFA-1, whereas CAM-70 does not [Hummel et al., 1998]. Thus, some strains of MV enhance expression of both ICAM-1 and one of its counterreceptors. The induction of ICAM-1 on endothelial cells by MV would be expected to lead to enhanced leukocyte adhesion and transendothelial migration, and this is consistent with the inflammatory infiltrates that have been observed near MV-infected endothelial cells in skin biopsies. However, the strain-specific differences in induction of ICAM-1 do not correlate with pathogenecity; thus, the functional consequences of ICAM-1 are difficult to ascertain. The expression of ICAM-1 by both cells expressing MV protein and neighboring cells that do not express MV protein likely recruits leukocytes that could help the host clear the infection. This hypothesis is supported by the work done by Soilu-Hanninen et al. [1996], which demonstrated that a greater percentage of monocytes bind to an MV-infected endothelial cell line than to uninfected cells.

Immunocompromised patients are less likely to develop a rash and are at increased risk for death from MV [Kaplan et al., 1992], suggesting that cellular adhesion molecule expression and induction of an inflammatory response that occurs in normal individuals is beneficial by helping to resolve the infection. Although the expression of ICAM-1 is a key component of the inflammatory response, its expression in HUVECs will not be a useful phenotypic marker of attenuation of MV strains because of the differential responses observed for two vaccine strains. Nonetheless, the induction of ICAM-1 on endothelial cells by Mor and the delayed weak induction by CAM-70 and Ed-wt could have important immunologic consequences and may effect viral clearance.

## REFERENCES

Adams MD, Dubnick M, Kerlavage AR, Moreno R, Kelley JM, Utterback TR, Nagle JW, Fields C, Venter JC. 1992. Sequence identification of 2,375 human brain genes. Nature 355:632–634.

Ades E, Candal F, Swerlick R, George V, Summers S, Bosse D, Lawley T. 1992. HMEC-1: establishment of an immortalized human microvascular endothelial cell line. J Invest Dermatol 99:683–690.

Attibele N, Wyde PR, Trial J, Smole SC, Smith CW, Rossen RD. 1993. Measles virus-induced changes in leukocyte function antigen 1 expression and leukocyte aggregation: possible role in measles virus pathogenesis. J Virol 67:1075–1079.

Bellini W, Englund G, Rammohan K, McFarlin DE. 1986. Evaluation of the structural proteins of the hamster neurotropic strain of measles virus with monoclonal antibodies. J Neuroimmunol 11: 149–163.

Bellini WJ, Rota JS, Rota PA. 1994. Virology of measles [review]. J Infect Dis 170:S15–S23.

Brankin B, Hart MN, Cosby SL, Fabry Z, Allen IV. 1995. Adhesion molecule expression and lymphocyte adhesion to cerebral endo-

thelium: effects of measles virus and herpes simplex 1 virus. J Neuroimmunol 56:1–8.

Caughman SW, Li LJ, Degitz K. 1990. Characterization and functional analysis of interferon-gamma-induced intercellular adhesion molecule-1 expression in human keratinocytes and A-431 cells. J Invest Dermatol 94:22S–26S.

Caughman SW, Li LJ, Degitz K. 1992. Human intercellular adhesion molecule 1 gene and its expression in the skin. J Invest Dermatol 96:61S–65S.

Collins T, Read MA, Neish AS, Whitley MZ, Thanos D, Maniatis T. 1995. Transcriptional regulation of endothelial cell adhesion molecules: NF-κB and cytokine-inducible enhancers. FASEB J 9:899–909.

Cornelius LA, Taylor JT, Degitz K, Li LJ, Lawley TJ, Caughman SW. 1993. A 5′ portion f the ICAM-1 gene confers tissue-specific differential expression levels and cytokine responsiveness. J Invest Dermatol 100:753–758.

Degitz K, Li LJ, Caughman SW. 1991. Cloning and characterization of the 5′-transcriptional regulatory region of the human intercellular adhesion molecule 1 gene. J Biol Chem 266:14024–14030.

Dustin ML, Springer TA. 1988. Lymphocyte function-associated antigen-1 (LFA-1) interaction with intercellular adhesion molecule-1 (ICAM-1) is one of at least three mechanisms for lymphocyte adhesion to cultured endothelial cells. J Cell Biol 107:221–331.

Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA. 1986. Induction by IL 1 and interferon-gamma: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J Immunol 137:245–254.

Enders JF, Peebles TC. 1954. Propagation in tissue cultures of cytopathogenic agents from people with measles. Proceedings of the Society of Experimental Biological Medicine 98:277–286.

Gellin BG, Katz SL. 1994a. Measles: state of the art and future directions [review]. J Infect Dis 170:S3–S14.

Gellin BG, Katz SL. 1994b. Putting a stop to a serial killer: measles. J Infect Dis 170:S1–S2.

Harcourt B, Rota P, Bellini W, Offermann MK. 1995. Differential induction of ICAM and IL-6 by two strains of measles virus in endothelial cells. J Interferon Cytokine Res 15:S231.

Hummel KB, Bellini WJ, Offermann MK. 1998. Strain-specific differences in LFA-1 induction on measles virus-infected monocytes and adhesion and viral transmission to endothelial cells. J Virol 72:8403–8407.

Kaplan LJ, Daum RS, Smaron M, McCarthy CA. 1992. Severe measles in immunocompromised patients. JAMA 267:1237–1241.

Kimura A, Tosaka K, Nakao T. 1975a. An immunofluorescent and electron microscopic study of measles skin eruptions. Tohoku J Exp Med 117:245–256.

Kimura A, Tosaka K, Nakao T. 1975b. Measles rash. I. Light and electron microscopic study of skin eruptions. Arch Virol 47:295–307.

Makgoba MW, Sanders ME, Ginther Luce GE, Dustin ML, Springer TA, Clark EA, Mannoni P, Shaw S. 1988. ICAM-1: a ligand for LFA-1-dependent adhesion of B, T and myeloid cells. Nature 331: 86–88.

Marui N, Offermann MK, Swerlick R, Kunsch C, Rosen CA, Ahmad M, Alexander RW, Medford RM. 1993. Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. J Clin Invest 92:1866–1874.

Offermann MK, Zimring J, Mellits KH, Hagen MK, Shaw R, Medford RM, Mathews MB, Goodbourn S, Jagus R. 1995. Activation of the double-stranded-RNA-activated protein kinase and induction of vascular cell adhesion molecule-1 by poly (I).poly (C) in endothelial cells. Eur J Biochem 232:28–36.

Rota JS, Wang ZD, Rota PA, Bellini WJ. 1994. Comparison of sequences of the H, F, and N coding genes of measles virus vaccine strains. Virus Res 31:317–330.

Selden RF. 1987. Analysis of RNA by Northern hybridization. In Ausubel FM, Brent R, Kingston RE, Moore DD, Smith JA, Seidman JG, Struhl K, editors. Current protocols in molecular biology. New York: John Wiley & Sons, p 4.9.1–4.9.8.

Simmons D, Makgoba MW, Seed B. 1988. ICAM, an adhesion ligand of LFA-1, is homologous to the neural cell adhesion molecule NCAM. Nature 331:624–627.

Soilu-Hanninen M, Hanninen A, Ilonen J, Salmi A, Salonen R. 1996. Measles virus hemagglutinin mediates monocyte aggregation and increased adherence to measles-infected endothelial cells. Med Microbiol Immunol 185:73–80.

Staunton DE, Marlin SD, Stratowa C, Dustin ML, Springer TA. 1988. Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families. Cell 52:925–933.

Swerlick RA, Lee KH, Li LJ, Sepp NT, Caughman SW, Lawley TJ. 1992. Regulation of vascular cell adhesion molecule 1 on human dermal microvascular endothelial cells. J Immunol 149:698–705.

Wyde PR, Attibele NR, Kemp WL. 1994. Infection of leukocytes by measles vaccine viruses Edmonston-Zagreb and Enders-Moraten has different consequences: potential mechanism for increase vaccine efficacy or aberrant activity in field trials. Vaccine 12:715–722.

Yang J, Xu Y, Zhu C, Hagan MK, Lawley T, Offermann MK. 1994. Regulation of adhesion molecule expression in Kaposi's sarcoma cells. J Immunol 152:361–373.

Zimring JC, Goodbourn S, Offermann MK. 1998. Human herpesvirus 8 encodes an interferon regulatory factor (IRF) homolog that represses IRF-1 mediated transcription. J Virol 72:701–707.